# Synthesis and evaluation of chromone-2-carboxamide derivatives as cytotoxic agents and 5-lipoxygenase inhibitors

**Fatima Bousejra-ElGarah[1] · Barbora Lajoie[1] · Jean-Pierre Souchard[2] · Geneviève Baziard[1] ·Jalloul Bouajila[2] ·Salomé El Hage[1]**

**Abstract** In the present study, we prepared a series of 21 chromone carboxamide derivatives bearing diverse amide side chains. Their potency to inhibit the proliferation of breast (MCF-7), ovarian (OVCAR and IGROV), and colon (HCT-116) cancer cell lines, was evaluated in vitro using the MTT assay. Among these compounds, 13 showed promising cytotoxic activity against at least one cancer cell line with $IC_{50}$ in the range 0.9–10 μM. Our compounds were also screened for their anti-inflammatory activity as putative inhibitors of 5-lipoxygenase.

Structure-activity relationships studies on our chromone carboxamide derivatives revealed that the presence of a 6-fluoro substituent on the chromone nucleus ($R_1$) or propyl and 3-ethylphenyl groups on the amide side chain ($R_2$) has a positive impact on the cytotoxic activity. In terms of the anti-inflammatory activity, hydrophilic chromone carboxamide derivatives showed greater 5-lipoxygenase inhibition.

The physico-chemical properties of chromone carboxamides are in accordance with the general requirements of drug development process and ligand efficiency values allow further structure optimization, with compound **4b** as a lead.

**Keywords** Chromone carboxamides · Cytotoxic activity · Anti-inflammatory effect · Lipophilicity · SAR

## Introduction

The chromone scaffold [(4H)-1-benzopyran-4-one] is well known as a pharmacophore of a large number of natural and synthetic bioactive molecules. This heterocycle constitutes the basic nucleus of flavones, an important and widespread class of compounds from plants with a large number of biological activities (Gaspar et al., 2014, Keri et al., 2014). Natural and synthetic chromones have shown a large spectrum of biological activities such as anti-inflammatory (Khan et al., 2010, Thanigaimalai et al., 2010), antimalarial (Auffret et al., 2007), and anticancer activity (Budzisz et al., 2002, Barve et al., 2006), associated with low toxicity (do Rocio Andrade Pires et al., 2016)

With 8.2 million deaths and 14.1 million new cases in 2012 (WHO, 2013), and given the fact that this number will reach 22.2 million by 2030, cancer has become one of the major health problems worldwide (Bray et al., 2012). The situation is expected to worsen because of the increase of multidrug resistances in various types of cancer (e.g., breast, ovarian, gastrointestinal, lung cancers) making the development of new active compounds a real challenge for the coming years (Pan et al., 2016).

In the search for new efficient anticancer drugs, chromones in particular carboxamides, can play an important

✉ Jalloul Bouajila
jalloul.bouajila@univ-tlse3.fr

✉ Salomé El Hage
salome.elhage@univ-tlse3.fr

[1] Laboratoire de Génie Chimique, Université de Toulouse, CNRS, INPT, UPS, Faculté des Sciences Pharmaceutiques, 35 chemin des Maraîchers, 31062 , Toulouse cedex 09, France

[2] Université de Toulouse 3 Paul-Sabatier, Faculté des Sciences Pharmaceutiques, Laboratoire des IMRCP UMR 5623 CNRS, 35 chemin des Maraîchers, 31062, Toulouse cedex 09, France

role as demonstrated by the growing interest toward this class of compounds. Indeed, several chromone carboxamides have been reported as potent and selective cytotoxic agents (Bhatnagar et al., 2010, Nam et al., 2010, Valdameri et al., 2012, Redda et al., 2014) and others were evaluated as monoamine oxidase inhibitors (Gaspar et al., 2011) or adenosine receptor ligands (Gaspar et al., 2012).

Besides, the association between cancer and inflammation has long been established (Balkwill and Mantovani, 2012). For instance, the involvement of the 5-lipoxygenase enzyme (5-LOX) and its products have been implicated in the development and progression of colon cancer (Soumaoro et al., 2006). 5-LOX has been reported to induce inflammation *via* the catalysis of the production of leukotrienes from arachidonic acid (Shimizu et al., 1984) and it is therefore an important target for mechanism-based drugs for the treatment of inflammatory disorders, often associated with other diseases including cancer. Therefore, anti-inflammatory agents that inhibit leukotriene production may have a beneficial effect in both the prevention and treatment of cancer (Aggarwal et al., 2006).

In the present study, we synthesized 21 substituted chromone carboxamides, among which 12 are newly described, and evaluated their potency to inhibit the proliferation of breast, ovarian, and colon cancer cell lines. Our compounds were also screened for their anti-inflammatory activity as putative inhibitors of 5-LOX. Additionally, an in silico study of the "drug-likeliness" properties for our derivatives was carried out by investigating their Lipinski parameters (Lipinski et al., 1997) and calculating their ligand efficiency values (Abad-Zapatero, 2007) to discuss structure–activity relationships (SAR).

## Materials and methods

### Chemistry

Melting points were determined on a DSC-50 Shimadzu apparatus (Kyoto, Japan). Infra-red spectra were recorded on a Perkin–Elmer Spectrum One fourier transform infrared spectrometer (Perkin–Elmer, USA). Proton nuclear magnetic resonance ($^1$H NMR) (300 MHz) and $^{13}$C NMR (75 MHz) spectra were obtained in $CDCl_3$ or DMSO-$d_6$ on a DPX 300 spectrometer (Brüker Biosciences, USA), and peak positions are given as s (singlet), d (doublet), t (triplet), q (quadruplet) or m (multiplet). Chemical shift ($\delta$) values are given in parts per million. Reactions were monitored by thin-layer chromatography (TLC) using pre-coated silica gel plates 60 F-254. All yields are calculated for analytically pure materials. The microanalyses were performed in the Microanalytical Laboratory of ENSIACET in Toulouse, France, and the results obtained were within ±0.4 % of the theoretical values. Mass spectra, electrospray ionization-method, were recorded on DSQ Thermo Fisher Scientific mass spectrometer; signals were given as *m/z*.

Commercially available chromone-2-carboxylic acids **1a**–**1e**, amine derivatives and all other reagents were purchased from Acros Organics (Halluin, France) and used without additional purification. Chemical structures were confirmed by infrared (IR), $^1$H NMR, $^{13}$C NMR, and ESI–MS.

The physico-chemical properties of compounds previously described **3a**, **3c**, **3d**, **3e** (Ellis and Shaw, 1972), **4a**, **5a** (Gaspar et al., 2011), **8a** (Gaspar et al., 2011), **9a** (Gaspar et al., 2012), and **10a** (Gaspar et al., 2012) are in agreement with the literature data.

*General procedure for the synthesis of acyl chloride 2*

4-oxo-4H-1-benzopyran-2-carbonyl chloride derivatives were prepared according to a previously described method (Payard, 1973). Phosphorus pentachloride (0.028 mol; 1.1 equiv.) was added to a solution of substituted chromone-2-carboxylic acid (0.025 mol) in dry cyclohexane (50 mL). The mixture was stirred under reflux for 1 h with the formation of the corresponding acyl chloride. After cooling, the formed solid was filtered and used without purification.

*General procedure for the synthesis of compounds **3–14***

An equimolar amount of the suitable acyl chloride was slowly added to a stirred solution of the corresponding amine (0.015 mol) in dry dichloromethane (DCM) (ammonium hydroxide, aniline, propylamine, 3-ethylaniline, 4-methylaniline, 4-chloroaniline, 4-fluoroaniline, 4-hydroxyaniline, 4-amino-α-diethylamino-*O*-cresol dihydrochloride, *N*,*N*-diethylpentyl-2,5-diamine, aminomorpholine, 1-(pyrrolidinocarbonylmethyl)piperazine) in presence of triethylamine (1 equiv.). The solution was stirred at room temperature and monitored by TLC. The mixture was washed with water, 10 % $Na_2CO_3$ and brine. Purification by flash silica gel chromatography (0–8 % MeOH in DCM) provided the desired amides.

6-Fluoro-4-oxo-4H-1-benzopyran-2-carboxamide (**3b**) Yield: 91 %; m.p.: 131 °C; IR (KBr) $\nu$ cm$^{-1}$: 3362 ($NH_2$), 3073, 3050, 2927 (CH), 1738, 1696 ($CONH_2$), 1635 (CO), 1624 (C=C); $^1$H NMR ($CDCl_3$): $\delta$ 6.95 (s, 1H, $H_3$), 7.80–7.94 (m, 3H, $H_5$, $H_7$, $H_8$), 8.33, and 8.70 (2s, 2H, $NH_2$); $^{13}$C NMR (DMSO-$d_6$) $\delta$: 177.30 ($C_4$), 161.02 ($C_6$), 158.45 ($C_2$), 156.51 (CO–$NH_2$), 152.04 ($C_{8a}$), 125.29 ($C_{4a}$), 123.47 ($C_7$), 122.16 ($C_8$), 110.17 ($C_3$), 109.88 ($C_5$); MS (*m/z*) 208.0 [M +H]$^+$. Anal. calcd. for $C_{10}H_6FNO_3$: C, 57.98; H, 2.92; N, 6.76 %. Found: C, 57.83; H, 2.97; N, 6.84 %.

6-Fluoro-*N*-propyl-4-oxo-4H-chromene-2-carboxamide (**4b**) Yield: 32 %; m.p.: 196 °C; IR (KBr) $\nu$ $cm^{-1}$: 3412 (NH), 3303, 2964, 2936, 2879 (CH, $CH_2$, $CH_3$), 1686 (CONH), 1642 (CO), 1618, 1540 (C=C); $^1$H NMR (DMSO-$d_6$): $\delta$ 0.91 (t, 3H, $CH_3$, $J$ = 7.5Hz), 1.57 (m, 2H, $CH_2$–C<u>H</u>$_2$–$CH_3$,), 3.26 (m, 2H, NH–$CH_2$), 6.83 (s, 1H, $H_3$), 7.71–7.86 (m, 3H, $H_5$, $H_7$, $H_8$), 9.14 (s br, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 177.25 ($C_4$), 160.89 ($C_6$), 158.45 ($C_2$), 156.46 (CO–NH), 152.00 ($C_{8a}$), 125.31 ($C_{4a}$), 123.72 ($C_7$), 122.14 ($C_8$), 110.15 ($C_3$), 109.91 ($C_5$), 41.47, 22.57 (2 $CH_2$), 11.83 ($CH_3$); MS (*m/z*) 250.0 $[M+H]^+$. Anal. calcd. for $C_{13}H_{12}FNO_3$: C, 62.65; H, 4.85; N, 5.62 %. Found: C, 62.76; H, 4.97; N, 5.46 %.

6-Bromo-*N*-propyl-4-oxo-4H-chromene-2-carboxamide (**4d**) Yield: 33 %; m.p.: 210 °C; IR (KBr) $\nu$ $cm^{-1}$: 3291 (NH), 3075, 2965, 2932, 2873 (CH, $CH_2$, $CH_3$), 1687 (CONH), 1646 (CO), 1600, 1536 (C=C); $^1$H NMR (DMSO-$d_6$): $\delta$ 0.90 (t, 3H, $CH_3$, $J$ = 7.4 Hz), 1.57 (m, 2H, $CH_2$–C<u>H</u>$_2$–$CH_3$), 3.26 (m, 2H, NH–C<u>H</u>$_2$), 6.85 (s, 1H, $H_3$), 7.71 (d, 1H, $H_8$, $J$ = 8.9 Hz), 8.06 (dd,1H, $H_7$, $J$ = 8.8, 2.5 Hz), 8.11 (d, 1H, $H_5$, $J$ = 2.5 Hz), 9.14 (s br, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 176.61 ($C_4$), 159.07 ($C_2$), 156.40 (CO–NH), 154.51 ($C_{8a}$), 138.03 ($C_7$), 127.48 ($C_5$), 125.57 ($C_{4a}$), 121.90 ($C_8$), 118.85 ($C_6$), 110.85 ($C_3$), 41.49, 22.58 (2 $CH_2$), 11.85 ($CH_3$); MS (*m/z*) 310.0 and 312 $[M+H]^+$. Anal. calcd. for $C_{13}H_{12}BrNO_3$: C, 50.34; H, 3.90; N, 4.52 %. Found: C, 50.45; H, 3.79; N, 4.63 %.

*N*-(3′-ethylphenyl)-4-oxo-4H-chromene-2-carboxamide (**6a**) Yield: 71 %; m.p.: 168 °C; IR (KBr) $\nu$ $cm^{-1}$: 3268 (NH), 2960, 2927, 2868 (CH, $CH_2$, $CH_3$), 1682 (CONH), 1640 (CO), 1615, 1540 (C=C); $^1$H NMR ($CDCl_3$) $\delta$: 1.29 (t, 3H, $CH_3$, $J$ = 7.6 Hz), 2.71 (q, 2H, $CH_2$, $J$ = 7.6 Hz), 7.09 (d, 1H, $H_8$, $J$ = 7.5 Hz), 7.32 (s,1H, $H_3$), 7.35 (t, 1H, $H_{5'}$, $J$ = 7.8 Hz), 7.49–7.64 (m, 4H, $H_6$, $H_{2'}$ $H_{4'}$, $H_{6'}$), 7.79 (td, 1H, $H_7$, $J$ = 7.2, 1.7 Hz), 8.27 (dd, 1H, $H_5$, $J$ = 7.9, 1.6 Hz), 8.54 (s br, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 177.79 ($C_4$), 158.08 ($C_2$), 156.19 (CO-NH), 155.62 ($C_{8a}$), 144.90 ($C_{3'}$), 137.99 ($C_{1'}$), 135.49 ($C_7$), 129.19 ($C_{5'}$), 126.56 ($C_6$), 125.39 ($C_5$), 124.94 ($C_{4'}$), 124.15 ($C_{4a}$), 120.89 ($C_{2'}$), 119.49 ($C_8$), 118.97 ($C_{6'}$), 111.48 ($C_3$), 28.68 ($CH_2$), 15.94 ($CH_3$); MS (*m/z*) 294.1 $[M+H]^+$. Anal. calcd. for $C_{18}H_{15}NO_3$: C, 73.71; H, 5.15; N, 4.78 %. Found: C, 73.59; H, 5.28; N, 4.92 %.

6-Fluoro-*N*-(3′-ethylphenyl)-4-oxo-4H-chromene-2-carboxamide (**6b**) Yield: 54 %; m.p.: 198 °C; IR (KBr) $\nu$ $cm^{-1}$: 3280 (NH), 3081, 2962, 2929, (CH, $CH_2$, $CH_3$), 1685 (CONH), 1637 (CO), 1612, 1593, 1543 (C=C); $^1$H NMR (DMSO-$d_6$): $\delta$ 1.21 (t, 3H, $CH_3$, $J$ = 7.6 Hz), 2.64 (q, 2H, $CH_2$, $J$ = 7.6 Hz), 6.98 (s, 1H, $H_3$), 7.06 (d, 1H, $H_{4'}$, $J$ = 7.6 Hz), 7.33 (t, 1H, $H_{5'}$, $J$ = 7.6 Hz), 7.64 (m, 2H, $H_{2'}$, $H_{6'}$), 7.77 (dd, 1H, $H_8$, $J$ = 8.3, 3.1 Hz), 7.85 (td, 1H, $H_7$, $J$ = 8.1, 3.1 Hz), 7.95 (dd, 1H, $H_5$, $J$ = 9.2, 4.4 Hz), 10.68 (s br, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 177.24 ($C_4$), 160.97 ($C_6$), 158.53 ($C_2$), 156.39 (CO-NH), 152.03 ($C_{8a}$), 144.94 ($C_{3'}$), 137.89 ($C_{1'}$), 129.22 ($C_{5'}$), 125.38 ($C_{4'}$), 125.31 ($C_{4a}$), 123.55 ($C_7$), 122.30 ($C_8$), 120.88 ($C_{2'}$), 118.96 ($C_{6'}$), 110.68 ($C_3$), 109.93 ($C_5$) 28.67 ($CH_2$), 15.94 ($CH_3$); MS (*m/z*) 312.1 $[M+H]^+$. Anal. calcd. for $C_{18}H_{14}FNO_3$: C, 69.45; H, 4.53; N, 4.50 %. Found: C, 69.57; H, 4.65; N, 4.39 %.

6-Bromo-*N*-(3′-ethylphenyl)-4-oxo-4H-chromene-2-carboxamide (**6d**) Yield: 52 %; m.p.: 227 °C; IR (KBr) $\nu$ $cm^{-1}$: 3267 (NH), 3075, 2961, 2960, 2927, (CH, $CH_2$, $CH_3$), 1683 (CONH), 1637 (CO), 1608, 1597, 1541 (C=C); $^1$H NMR (DMSO-d6): $\delta$ 1.34 (t, 3H, $CH_3$, $J$ = 7.6 Hz), 2.76 (q, 2H, $CH_2$, $J$ = 7.6 Hz), 7.13 (s, 1H, $H_3$), 7.19 (d, 1H, $H_4'$, $J$ = 7.6 Hz), 7.46 (t, 1H, $H_5'$, $J$ = 7.7 Hz), 7.75 (m, 2H, $H_2'$, $H_6'$), 7.96 (d, 1H, $H_8$, $J$ = 8.9 Hz), 8.22 (dd, 1H, $H_7$, $J$ = 8.9, 2.5 Hz), 8.28 (d, 1H, $H_5$, $J$ = 2.5 Hz), 10.4 (s br, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 176.68 ($C_4$), 157.80 ($C_2$), 156.38 (CO–NH), 154.58 ($C_{8a}$), 144.95 ($C_3'$), 138.16 ($C_7$), 137.88 ($C_1'$), 129.23 ($C_5'$), 127.49 ($C_5$), 125.63 ($C_{4a}$), 125.04 ($C_4'$), 122.17 ($C_8$), 120.88 ($C_2'$), 119.04 ($C_6$), 118.97 ($C_6'$), 111.51 ($C_3$), 28.67 ($CH_2$), 15.94 ($CH_3$); MS (*m/z*) 372.0 and 374.0 $[M+H]^+$. Anal. calcd. for $C_{18}H_{14}BrNO_3$: C, 58.08; H, 3.79; N, 3.76 %. Found: C, 58.29; H, 3.64; N, 3.89 %.

*N*-(3′-ethylphenyl)-6-methyl-4-oxo-4H-chromene-2-carboxamide (**6e**) Yield: 33 %; m.p.: 202 °C; IR (KBr) $\nu$ $cm^{-1}$: 3326 (NH), 3074, 2928, 2850, (CH, $CH_2$, $CH_3$), 1681 (CONH), 1637 (CO), 1610, 1594, 1541 (C=C); $^1$H NMR (DMSO-$d_6$): $\delta$ 1.21 (t, 3H, $CH_2$-C<u>H</u>$_3$, $J$ = 7.6 Hz), 2.48 (s, 3H, $CH_3$), 2.64 (q, 2H, $CH_2$, $J$ = 7.6 Hz), 6.94 (s, 1H, $H_3$), 7.07 (d, 1H, $H_4'$, $J$ = 8.0 Hz), 7.33 (t, 1H, $H_5'$, $J$ = 8.0 Hz), 7.65 (m, 2H, $H_2'$, $H_6'$), 7.75 (m, 2H, $H_7$, $H_8$), 7.87 (s, 1H, $H_5$), 10.66 (s br, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 177.72 ($C_4$), 158.13 ($C_2$), 156.04 (CO–NH), 153.88 ($C_{8a}$), 144.89 ($C_3'$), 137.99 ($C_7$), 136.56 ($C_1'$), 136.29 ($C_6$), 129.18 ($C_5'$), 124.92 ($C_4'$), 124.62 (C5), 123.86 ($C_{4a}$), 120.86 ($C_2'$), 119.27 ($C_8$), 118.94 ($C_6'$), 111.31 ($C_3$), 28.68 ($CH_2$), 20.94 and 15.94 (2 $CH_3$); MS (*m/z*) 308.1 $[M+H]^+$. Anal. calcd. for $C_{19}H_{17}NO_3$: C, 74.25; H, 5.58; N, 4.56 %. Found: C, 74.37; H, 5.42; N, 4.69 %.

*N*-(4′-fluorophenyl)-4-oxo-4H-chromene-2-carboxamide (**7a**) Yield: 48 %; m.p.: 222 °C; IR (KBr) $\nu$ $cm^{-1}$: 3338 (NH), 3138, 3072 (CH), 1697 (CONH), 1630 (CO), 1573, 1508 (C=C); $^1$H NMR (DMSO-$d_6$): $\delta$ 6.98 (s,1H, $H_3$), 7.27 (m, 2H, $H_3'$, $H_5'$), 7.57 (m, 1H, $H_6$), 7.82–7.85 (m, 4H, $H_7$, $H_8$, $H_2'$, $H_6'$), 8.08 (dd, 1H, $H_5$, $J$ = 7.9, 1.6 Hz), 10.82 (s,1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 177.73 ($C_4$), 160.66 ($C_4'$), 158.25 ($C_2$), 156.01 (CO–NH), 155.60 ($C_{8a}$), 135.48 ($C_7$), 134.35 ($C_1'$), 126.55 ($C_6$), 125.39 ($C_5$), 124.14 ($C_{4a}$), 123.60 ($C_6'$), 123.52 ($C_2'$), 119.47 ($C_8$), 116.03 ($C_5'$), 115.80 ($C_3'$), 111.56 ($C_3$); MS (*m/z*) 284.0 $[M+H]^+$. Anal.

calcd. for $C_{16}H_{10}FNO_3$: C, 67.84; H, 3.56; N, 4.94 %. Found: C, 67.53; H, 3.62; N, 4.88 %.

*N*-[3-((diethylamino)methyl)-4-hydroxyphenyl]-4-oxo-4H-chromene-2-carboxamide (**11a**) Yield: 26 %; m.p.: 186 °C; IR (KBr) $\nu$ $cm^{-1}$: 3470 (OH), 3339 (NH), 3075, 2975, 2827 (CH, $CH_2$, $CH_3$), 1685 (CONH), 1638 (CO), 1616, 1560, 1512 (C=C); $^1$H NMR (DMSO-$d_6$): $\delta$ 1.05 (t, 6H, $CH_2$–C<u>H</u>$_3$, $J = 6.8$ Hz), 2.56 (m, 4H, N–C<u>H</u>$_2$–$CH_3$), 3.33 (s br, 1H, OH), 3.73 (s, 2H, Ar–C<u>H</u>$_2$–N), 6.73 (d, 1H, $H_5'$, $J = 8.4$ Hz), 6.94 (s, 1H, $H_3$), 7.54 (m, 2H, $H_6$ and $H_6'$), 7.70 (s, 1H, $H_2'$), 7.85 (d, 1H, $H_8$, $J = 8.7$ Hz), 7.93 (t, 1H, $H_7$, $J = 7.5$ Hz), 8.08 (d, 1H, $H_5$, $J = 6.9$ Hz), 10.56 (s br, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 177.92 ($C_4$), 157.70 ($C_2$), 156.36 (CO–NH), 155.61 ($C_{8a}$), 154.92 ($C_4'$), 135.54 ($C_7$), 129.32 ($C_1'$), 129.30 ($C_6'$), 126.52 ($C_6$), 125.39 ($C_5$), 125.37 ($C_3'$), 124.07 ($C_{4a}$), 123.29 ($C_2'$), 119.48 ($C_8$), 115.91 ($C_5'$), 111.27 ($C_3$), 53.19 (Ar–$CH_2$–N), 46.65 (N–$CH_2$), 10.51 ($CH_3$); MS (*m/z*) 367.2 $[M+H]^+$. Anal. calcd. for $C_{21}H_{22}N_2O_4$: C, 68.84; H, 6.05; N, 7.65 %. Found: C, 68.92; H, 6.24; N, 7.52 %.

N-(5-(diethylamino)pent-2-yl)-4-oxo-4H-chromene-2-carboxamide (**12a**) Yield: 57 %; m.p.: 110 °C; IR (KBr) $\nu$ $cm^{-1}$: 3475, 3428, 3370 (NH), 2978, 2939, 2633 (CH, $CH_2$, $CH_3$), 1679 (CONH), 1647 (CO), 1608, 1572, 1528 (C=C); $^1$H NMR (DMSO-$d_6$): $\delta$ 1.19 (t, 6H, $CH_2$–C<u>H</u>$_3$, $J = 7.2$ Hz), 1.23 (d, 3H, CH–C<u>H</u>$_3$, $J = 6.6$ Hz), 1.62 (m, 4H, CH–C<u>H</u>$_2$–C<u>H</u>$_2$–$CH_2$–N), 3.05 (m, 6H, 3 $CH_2$–N), 4.05 (m, 1H, CH), 6.85 (s, 1H, $H_3$), 7.55 (td, 1H, $H_6$, $J = 7.9$, 0.9 Hz), 7.82 (d, 1H, $H_8$, $J = 7.8$ Hz), 7.91 (td, 1H, $H_7$, $J = 8.5$, 1.6 Hz), 8.05 (dd, 1H, $H_5$, $J = 7.9$, 1.3 Hz), 10.13 (s br, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 177.86 ($C_4$), 159.02 ($C_2$), 156.27 (CO-NH), 155.60 ($C_{8a}$), 135.38 ($C_7$), 126.49 ($C_6$), 125.30 ($C_5$), 124.05 ($C_{4a}$), 119.51 ($C_8$), 110.97 ($C_3$), 50.76 and 46.50 (2 $CH_2$), 45.42 (CH), 32.96 ($CH_2$), 20.89 ($CH_3$) 20.48 ($CH_2$), 8.82 ($CH_3$); MS (*m/z*) 331.2 $[M+H]^+$. Anal. calcd. for $C_{19}H_{26}N_2O_3$: C, 69.06; H, 7.93; N, 8.48 %. Found: C, 69.23; H, 7.72; N, 8.33 %.

N-morpholino-4-oxo-4H-chromene-2-carboxamide (**13a**) Yield: 57 %; m.p.: 271 °C; IR (KBr) $\nu$ $cm^{-1}$: 3219 (NH), 3061, 2962, 2938, 2874 (CH), 1684 ($CONH_2$), 1644 (CO), 1620, 1607, 1511 (C=C); $^1$H NMR (DMSO-$d_6$): $\delta$ 2.94 (t, 4H, O–$CH_2$–C<u>H</u>$_2$–N, $J = 4.6$ Hz), 3.69 (t, 4H, O–C<u>H</u>$_2$–$CH_2$–N, $J = 4.6$ Hz), 6.82 (s, 1H, $H_3$), 7.55 (td, 1H, $H_6$, $J = 8.0$, 1.0 Hz), 7.76 (d, 1H, $H_8$, $J = 7.9$ Hz), 7.90 (m, 1H, $H_7$), 8.05 (dd, 1H, $H_5$, $J = 7.9$, 1.6 Hz), 10.20 (s br, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 177.69 ($C_4$), 157.30 ($C_2$), 156.15 (CO–NH), 155.57 ($C_{8a}$), 135.51 ($C_7$), 126.57 ($C_6$), 125.39 ($C_5$), 124.09 ($C_{4a}$), 119.34 ($C_8$), 111.25 ($C_3$), 66.39 (2 $CH_2$), 54.63 (2 $CH_2$); MS (*m/z*) 275.0 $[M+H]^+$. Anal. calcd. for $C_{14}H_{14}N_2O_4$: C, 61.31; H, 5.14; N, 10.21 %. Found: C, 61.45; H, 5.06; N, 10.36 %.

2-[4(*N*-pyrrolidinylcarbonylmethyl)-piperazinylcarbonyl]-4H-chromen-4-one (**14a**) Yield: 55 %; m.p.: 117 °C; IR (KBr) $\nu$ $cm^{-1}$: 3052, 2973, 2955, 2867, 2820 (CH, $CH_2$, $CH_3$), 1662 (CONH), 1644 (CO), 1617, 1573, 1510 (C=C); $^1$H NMR ($CDCl_3$): $\delta$ 1.89 and 1.98 (2m, 4H, $H_3''$, $H_4''$), 2.7 (m, 4H, $H_2''$, $H_5''$), 3.22 (s, 2H, N–$CH_2$–CO–), 3.48 (m, 4H, $H_3'$, $H_5'$), 3.62, and 3.85 (2m, 4H, $H_2'$, $H_6'$), 6.53 (s, 1H, $H_3$), 7.46 (t, 1H, $H_6$, $J = 7.1$ Hz), 7.52 (d, 1H, $H_8$, $J = 8.6$ Hz), 7.76 (td, 1H, $H_7$, $J = 7.1$, 1.7 Hz), 8.25 (dd, 1H, $H_5$, $J = 8.0$, 1.5 Hz); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 177.08 ($C_4$), 167.66 (CO–$CH_2$), 160.52 (CO–NH), 158.46 ($C_2$), 155.91 ($C_{8a}$), 135.51 ($C_7$), 126.57 ($C_6$), 125.39 ($C_5$), 124.16 ($C_{4a}$), 119.08 ($C_8$), 111.01 ($C_3$), 60.22 (CO–C<u>H</u>$_2$–N), 53.06 and 52.23 (2C pyrrolidine), 46.99, 45.99, 45.81 and 42.20 (4C piperazine), 26.16 and 24.12 (2C pyrrolidine); MS (*m/z*) 370.2 $[M+H]^+$. Anal. calcd. for $C_{20}H_{23}N_3O_4$: C, 65.03; H, 6.28; N, 11.37 %. Found: C, 65.21; H, 6.15; N, 11.25 %.

## Pharmacology

### *Anti-inflammatory activity*

The anti-inflammatory activity of compounds was determined by the inhibition of soybean lipoxygenase. Chromone derivatives were dissolved in dimethyl sulfoxide (DMSO) and solutions were diluted in sodium phosphate buffer (pH 7.4). Diluted solutions of compounds (20 µL) were mixed individually with 30 µL of sodium phosphate buffer containing 5-LOX and 60 µL of linoleic acid (3.5 mM) in a 96-well microliter plate. Addition of 90 µL buffer gave a total volume of 200 µL and a final concentration of chromone carboxamide of 100 µM. The DMSO concentration did not exceed 1 %. The mixture was incubated at 25 °C for 10 min, and the absorbance was recorded at 234 nm using a Thermo Scientific Multiskan GO Microplate Spectrophotometer. The absorption changes with the conversion of linoleic acid to 13-hydroperoxyoctadeca-9,11-dienoate. Nordihydroguaiaretic acid (NDGA) was used as positive control. Percentage inhibition of enzyme was determined by comparison of rates of reaction of samples relative to blank sample using the formula $(E–S)/E \times 100$, where $E$ is the activity of enzyme without test sample and $S$ is the activity of enzyme with test sample. The percentage of inhibition of each compound was determined in triplicate.

### *Cytotoxicity evaluation*

The cytotoxicity of compounds was estimated on human cancer cell lines (MCF-7, HCT-116, OVCAR, IGROV) using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. Cells were distributed in 96-well plates at $3 \times 10^4$ cells/well in 100 µL culture medium, and then 100 µL of medium containing the chromone

carboxamide at various concentrations were added. MTT is a yellow water-soluble tetrazolium salt, which metabolically active cells convert to water-insoluble dark blue formazan by reductive cleavage of the tetrazolium ring. All compounds were dissolved in DMSO followed by dilution in buffer so that the DMSO concentration did not exceed 1 %. The level of blue formazan is then used as indirect index of cell density. The optical density of each sample was measured with a Thermo Scientific Multiskan GO Microplate Spectrophotometer at 500 nm. Four replicates were performed for each sample. Tamoxifen was used as positive control.

## Results and discussion

### Chemistry

Chromone carboxamides derivatives were obtained by functionalization of the chromone nucleus at position C2 of the γ-pyrone ring.

Structural modifications have been made in order to evaluate the SAR on cytotoxicity and 5-LOX inhibition. Particularly, (i) the introduction of a halide or methyl group on the 6-position of the chromone nucleus (R1, scheme 1), (ii) the side chain nature, either aliphatic or aromatic (R2), (iii) the substitution on the aromatic moiety, and (iv) the functionalization of the hydroxyl group have been considered. The molecular diversity in this library of compounds was further increased by preparing analogs **12a**, **13a,** and **14a** bearing nitrogen-containing side chains.

Syntheses were carried out starting from 6-substituted 2-chromone carboxylic acids, prepared according to a previously described method (Payard et al., 1974). The carboxamide derivatives were prepared in a two-step process, involving first the conversion, in quantitative yield, of the carboxylic acid into the acylchloride **2** using $PCl_5$ (Payard, 1973), and then the condensation with the appropriate amine to give amides **3**–**14** (scheme 1). The main advantage of this synthetic procedure is that no coupling reagents are needed. Chromone carboxamides were synthesized in a range of 30–90 % yields, similar to those reported with the use of coupling agents such as phosphonium salts (BOP or PyBOP) (Gaspar et al., 2011). Their chemical structures were confirmed by IR, $^{1}H$ NMR, $^{13}C$ NMR and ESI–MS.

Reactions conditions: i) $PCl_5$/dry cyclohexane; ii) $R_2$-$NH_2$/TEA/dry DCM.

### Pharmacology

#### *Cytotoxicity*

Twenty one chromone carboxamide derivatives were assayed for their in vitro anti-proliferative effect against four human cancer cell lines: MCF-7 (hormone dependent breast cancer), OVCAR and IGROV (ovary cancer) and HCT-116 (colon cancer) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay as described by Mosmann (1983) with modifications (Bekir et al., 2013). The results, expressed as $IC_{50}$ values (μM), i.e., the concentration required to inhibit cell proliferation by 50 % after exposure of cells to test compounds, are summarized in Table 1 and tamoxifen was used as reference.

Among these compounds, 13 showed good cytotoxic activity against at least one cancer cell line ($IC_{50} \leq 10$ μM). In the case of the breast cancer cell line MCF-7, five compounds were active with the best antiproliferative activity observed for **4b** ($IC_{50} = 0.9$ μM). Eight compounds were active against OVCAR cells (lowest $IC_{50}$ for **4b** and **11a** = 5.1 μM), eight derivatives were cytotoxic against IGROV cells (lowest $IC_{50}$ for **4b** and **10a** = 3.2 μM), and four compounds were active against colon HTC-116 cells (lowest $IC_{50}$ for **4d** = 4.5 μM).

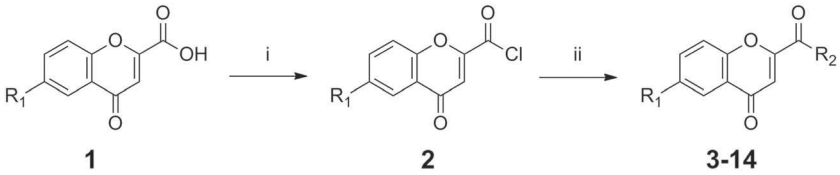
The tests showed that the primary amide analogs **3a**–**3e** are not cytotoxic, except the 6-fluorinated compound **3b,** which had an interesting activity against the two cancer cell lines OVCAR and IGROV ($IC_{50} = 10.1$ and 11.8 μM, respectively).

Amide alkylation by a propyl side chain (compounds **4a**–**4d**) had a significantly positive impact on the cytotoxic activity. For instance the 6-fluorinated propyl carboxamide **4b** ($IC_{50}$ range: 0.9–22.2 μM) was more cytotoxic than its primary analog **3b** ($IC_{50}$ range: 10.1–44.2 μM). Compound **4b** was also the most potent compound against hormone dependent breast cancer cells MCF-7 with an $IC_{50}$ value in the same range as tamoxifen ($IC_{50} = 0.9$ and 0.39 μM, respectively). Regarding the non-substituted chromone series ($R_1$ = H), alkylation by a 3-ethylphenyl ring also had a positive impact as compound **6a** exhibited $IC_{50}$ values against the four cell lines in the same range as the propyl carboxamide derivative **4a** ($IC_{50}$ range: 5.3–18.0 μM). However, the positive effect of the 6-fluoro substituent on activity showed with compounds **3b** and **4b** was not observed with derivative **6b**, less active than its 6-brominated and 6-methylated analogs (**6d** and **6e**).

The beneficial effect of fluorine seen with the 6-fluoro chromone series was not observed when the fluorine atom was placed on the phenylcarboxamide side chain (compound **7a**) as the toxicity towards the tested cell lines was not enhances compared to the chloro (**8a**) and methyl (**9a**) analogs.

The presence of a hydroxyl group in the *para* position of the phenylcarboxamide produced a beneficial effect as compound **10a** showed $IC_{50}$ values from 3.2 to 12.3 μM. On adding a methyldiethylamino chain in the *meta* position (compound **11a**), only the activity against OVCAR cells was conserved ($IC_{50} = 5.1$ μM).

**Scheme 1** Synthesis of compounds **3–14**




Reactions conditions: i) $PCl_5$/dry cyclohexane; ii) $R_2$-$NH_2$/TEA/dry DC

| | $R_1$ | $R_2$ |
|---|---|---|
| **3a** | H | -$NH_2$ |
| **3b** | F | -$NH_2$ |
| **3c** | Cl | -$NH_2$ |
| **3d** | Br | -$NH_2$ |
| **3e** | $CH_3$ | -$NH_2$ |
| **4a** | H | -NH-$C_3H_7$ |
| **4b** | F | -NH-$C_3H_7$ |
| **4d** | Br | -NH-$C_3H_7$ |
| **5a** | H | -NH-Ph |
| **6a** | H | -NH-(3'-$C_2H_5$)-Ph |
| **6b** | F | -NH-(3'-$C_2H_5$)-Ph |
| **6d** | Br | -NH-(3'-$C_2H_5$)-Ph |
| **6e** | $CH_3$ | -NH-(3'-$C_2H_5$)-Ph |
| **7a** | H | -NH-(4'-F)-Ph |
| **8a** | H | -NH-(4'-Cl)-Ph |
| **9a** | H | -NH-(4'-$CH_3$)-Ph |
| **10a** | H | -NH-(4'- OH)-Ph |
| **11a** | H | -NH-(4'-OH, 3'-$CH_2$-N($C_2H_5$)$_2$)-Ph |
| **12a** | H | -NH-CH($CH_3$)-($CH_2$)$_3$-N($C_2H_5$)$_2$ |
| **13a** | H | -NH-morpholino |
| **14a** | H | -N-2-(4-pyrrolidinylcarbonylmethyl)-piperazinyl |

To widen the panel of structures, we also studied chromone carboxamides bearing various non-aromatic nitrogen-containing side chains: *N*,*N*-(diethyl)pentanamine, morpholine and *N*-substituted piperazine (**12a**–**14a**). The impact of these groups on the activity was not the same depending on the cell line but one can note that the $IC_{50}$ values were kept below 10 μM against at least one cell line.

Overall, while several compounds in the present library possess $IC_{50}$ values below 10 μM, none of them displays good selectivity, i.e., activity against one cell line 10-fold higher than the three other cell lines.

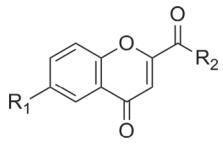
*Anti-inflammatory activity*

In order to study the ability of compounds **3**–**14** to directly inhibit the catalytic activity of 5-LOX, a cell-free assay using purified 5-LOX enzyme in the presence of 100 μM of the chromone derivative was applied, according to the method described by Baylac and Racine (Baylac and Racine, 2003) with modifications. Among 20 chromone carboxamide derivatives tested as putative inhibitors of the 5-LOX enzyme, 10 were totally inactive or showed low activity (<20 %) and 10 showed moderate to good activity (Table 1).

The 6-fluorinated compound **3b** showed interesting inhibition (79.9 %). Here again, the effect of a 6-fluoro substituent was positive for 5-LOX inhibition compared to its 6-Cl, 6-Br, and 6-methyl analogs (**3c**–**3e**). The presence of the propyl side chain (**4b**) led to a decreased activity (68.0 % instead of 79.9 % for **3b**).

Alkylation with a phenyl moiety, either substituted or not with ethyl, fluoro, chloro, or methyl groups (compounds **6a**–**e**, **7a**–**9a**) had a major negative impact on the anti-inflammatory activity. However, using a *para* phenol as alkylating group (compound **10a**) had a positive effect on the anti-inflammatory activity as 5-LOX inhibition increased up to 73.5 % compared to 55.2 % for the primary amide homolog **3a**. The beneficial effect of a *para* phenol

**Table 1** In vitro cytotoxicity against four human cancer cell lines and anti-inflammatory activity of compounds **1**, **3–14**



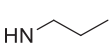
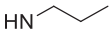
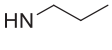
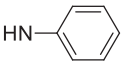
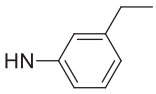
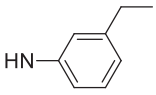
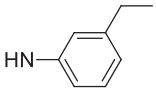
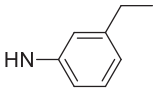
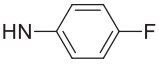
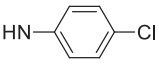
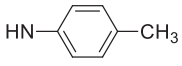
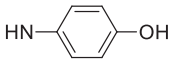
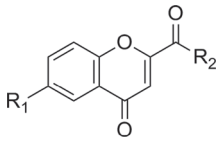
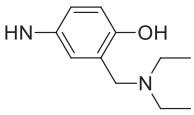
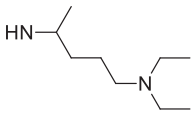
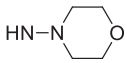
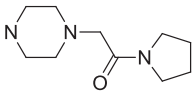

| | | | Antiproliferative activity $IC_{50}$ (μM) | | | | Anti-inflammatory activity % inhibition (100 μM) |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | MCF-7 | OVCAR | IGROV | HCT-116 | 5-Lipoxygenase |
| **1a** | H | OH | >100 | >100 | 20.2 ± 4.1 | 14.4 ± 1.3 | ND |
| **3a** | H | $NH_2$ | >100 | >100 | >100 | >100 | 55.2 ± 4.0 |
| **3b** | F | $NH_2$ | 44.2 ± 5.0 | 10.1 ± 1.3 | 11.8 ± 1.3 | 33.2 ± 2.2 | 79.9 ± 6.6 |
| **3c** | Cl | $NH_2$ | 89.2 ± 13.2 | >100 | >100 | >100 | 41.8 ± 6.1 |
| **3d** | Br | $NH_2$ | >100 | >100 | >100 | >100 | 15.5 ± 1.2 |
| **3e** | $CH_3$ | $NH_2$ | 80.2 ± 13.2 | >100 | >100 | >100 | 12.9 ± 0.6 |
| **4a** | H |  | 11.2 ± 1.1 | 10.1 ± 1.1 | 5.4 ± 0.3 | 20.2 ± 1.1 | 67.2 ± 3.4 |
| **4b** | F |  | 0.9 ± 0.0 | 5.1 ± 0.3 | 3.2 ± 0.1 | 22.2 ± 2.2 | 68.0 ± 1.8 |
| **4d** | Br |  | 12.6 ± 1.0 | 25.2 ± 2.3 | 7.2 ± 0.4 | 4.5 ± 0.2 | 30.5 ± 5.1 |
| **5a** | H |  | >100 | >100 | >100 | >100 | ND |
| **6a** | H |  | 5.3 ± 0.6 | 8.0 ± 0.4 | 18.0 ± 2.0 | 14.2 ± 0.9 | NA |
| **6b** | F |  | 56.1 ± 6.4 | 5.8 ± 0.3 | 13.2 ± 1.2 | 32.3 ± 4.4 | NA |
| **6d** | Br |  | 6.3 ± 0.4 | 20.1 ± 3.3 | 8.0 ± 1.2 | >100 | NA |
| **6e** | $CH_3$ |  | 18.0 ± 2.2 | 9.2 ± 1.3 | 5.9 ± 0.0 | >100 | NA |
| **7a** | H |  | 63.2 ± 5.3 | >100 | 18.0 ± 2.1 | 25.2 ± 3.2 | NA |
| **8a** | H |  | >100 | >100 | 22.1 ± 1.2 | 9.0 ± 0.4 | NA |
| **9a** | H |  | >100 | >100 | 25.4 ± 2.2 | 11.3 ± 1.2 | NA |
| **10a** | H |  | 12.3 ± 1.0 | 6.3 ± 0.2 | 3.2 ± 0.0 | 9.0 ± 1.3 | 73.5 ± 3.4 |

**Table 1** continued



| | $R_1$ | $R_2$ | Antiproliferative activity $IC_{50}$ (μM) | | | | Anti-inflammatory activity % inhibition (100 μM) |
|---|---|---|---|---|---|---|---|
| | | | MCF-7 | OVCAR | IGROV | HCT-116 | 5-Lipoxygenase |
| **11a** | H |  | 34.8 ± 3.2 | 5.1 ± 0.3 | 12.0 ± 0.4 | 40.0 ± 3.0 | 52.0 ± 4.9 |
| **12a** | H |  | 40.2 ± 2.0 | 10.4 ± 1.2 | 6.8 ± 0.4 | 36.2 ± 3.1 | 48.6 ± 2.3 |
| **13a** | H |  | 8.3 ± 0.1 | 25.3 ± 2.4 | 13.1 ± 1.2 | 5.8 ± 1.1 | 68.8 ± 4.6 |
| **14a** | H |  | 6.0 ± 0.5 | 28.0 ± 2.2 | 10.1 ± 0.2 | 31.3 ± 3.3 | 62.3 ± 2.9 |
| Tamoxifen | | | 0.39 ± 0.01 | 0.52 ± 0.04 | 0.82 ± 0.10 | 0.37 ± 0.02 | — |
| NDGA (13 μM) | | | — | — | — | — | 50.0 ± 2.7 |

*NDGA* nordihydroguaiaretic acid, *NA* not active, *ND* not determined

was reduced when a *N*-diethylmethyl side chain was added in the *meta* position of the phenyl group (52.0 % for compound **11a**).

Amidation using other nitrogen containing side chains (*N*-diethylpentyl **12a**, morpholinyl **13a**, and substituted piperazinyl **14a**) showed good activity with 5-LOX inhibition from 48.6 % up to 68.8 %.

## Theoretical evaluation of "drug-likeliness" properties

To predict their overall "drug-likeness", an in silico computational study of the synthesized chromone carboxamides **3**–**14** was performed by determining Lipinski parameters (Lipinski et al., 1997) using the molinspiration online software (www.molinspiration.com) (Table 1 of supplementary material). To better understand the therapeutic potential of chromone derivatives, we also calculated their ligand efficiency (LE) for all the cell lines (Table 2 of supplementary material). LE is a measure of the in vitro biological activity corrected for the physico-chemical properties of the molecule and quantify how effectively the molecule uses its structural features in binding the target. LE is therefore a useful metric to normalize the affinities of hits to identify the best starting points for optimization, i.e., those with the highest LE values (Hopkins et al., 2014).

From the data obtained, one can observe that all the chromone derivatives possess LogP values compatible with those required to cross membranes and no violations of Lipinski's rules were found (Table 2), thus making our chromone derivatives promising therapeutic agents with a good potential for eventual development as orally active drug candidates. Lipophilicity does seem to play a significant role for 5-LOX inhibition as, except for **11a**, derivatives with logP ≥ 3.01 were totally inactive (compounds **6a**–**9a**) and the six most potent chromone carboxamides had a logP value < 2.47.

Overall, the lowest $IC_{50}$ value was displayed by compound **4b** against MCF-7 cell line. In order to see if this compound is the one which should be selected as a lead for further investigations, we calculated the LE values for all compounds against this cell line. Results showed that LE values are in the range 0.36–0.46 kcal/mol/non-H atom.

**Table 2** LogP and LE values of the chromone derivatives

| N° | LogP[a] | MW | $IC_{50}$ (μM) | $LE_{MCF-7}$[b] |
|---|---|---|---|---|
| **3b** | 0.04 | 207.16 | 44.2 | 0.40 |
| **3c** | 1.53 | 223.61 | 89.2 | 0.37 |
| **3e** | 1.30 | 203.19 | 80.2 | 0.38 |
| **4a** | 2.13 | 231.25 | 11.2 | 0.40 |
| **4b** | 1.30 | 249.24 | 0.9 | 0.46 |
| **4d** | 2.91 | 310.14 | 12.6 | 0.38 |
| **6a** | 3.84 | 293.32 | 5.3 | 0.33 |
| **6b** | 3.01 | 311.31 | 56.1 | 0.26 |
| **6d** | 4.62 | 372.21 | 6.3 | 0.31 |
| **6e** | 4.26 | 307.34 | 18.0 | 0.28 |
| **7a** | 3.11 | 283.25 | 63.2 | 0.28 |
| **10a** | 2.47 | 281.26 | 12.3 | 0.32 |
| **11a** | 3.42 | 366.41 | 34.8 | 0.23 |
| **12a** | 2.90 | 330.42 | 40.2 | 0.25 |
| **13a** | 0.09 | 274.27 | 8.3 | 0.35 |
| **14a** | 1.14 | 369.41 | 6.0 | 0.27 |

[a] LogP values were determined with molinspiration software (www.molinspiration.com)

[b] Ligand efficiency (LE) was calculated from the equation $LE = 1.4 (-\log(IC_{50})/N)$ where "$IC_{50}$" is the molar concentration required to inhibit MCF-7 cells proliferation by 50 % and "N" is the number of non-hydrogen atoms

The highest LE value (0.46 kcal/mol/non-H atom) was observed for compound **4b**. In addition, this compound also exhibits good LE values for the three other cell lines (0.36–0.42 kcal/mol/non-H atom). As compound **4b** is relatively small, this result means that this molecule can be further improved for enhanced potency.

## Conclusion

In this study, 21 chromone carboxamide derivatives were synthesized using simple and affordable chemistry and evaluated for anti-proliferative activity against four human cancer cells. Among these compounds, 13 showed $IC_{50}$ values below 10 μM against at least one cancer cell line. Compound **4b** was the most promising one with cytotoxic effect against breast cancer cell line MCF-7 equivalent to tamoxifen's. Among the four cancer cell lines, the ovarian IGROV line was the most sensitive to the tested compounds. Chromone carboxamide derivatives with a 6-fluoro substituent on the chromone nucleus ($R_1$) or propyl or 3-ethylphenyl groups on the amide side chain ($R_2$) showed better cytotoxic effect. In terms of anti-inflammatory activity, hydrophilic chromone carboxamide derivatives showed greater 5-LOX inhibition.

Overall, the physico-chemical properties (lipophilicity, molecular weight, number of H acceptors/donnors) of chromone carboxamides are in accordance with the general requirements of drug discovery and development process and LE values allow further structure optimization, with compound **4b** as a lead. Other compounds such as **6a** or **10a** are also promising compounds. Therefore, this study provides a solid base for further research on chromone carboxamides to get more potent and selective cytotoxic agents.

**Acknowledgments** We are thankful to Laurent Amielet, Christelle Récochet-Gueriot and Sylvie Cazaux for technical support. We are grateful to the "VLM" association for financial support.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no competing interests.

## References

Abad-Zapatero C (2007) Ligand efficiency indices for effective drug discovery. Expert Opin Drug Dis 2:469–488

Aggarwal BB, Shishodia S, Sandur SK, Pandey MK, Sethi G (2006) Inflammation and cancer: How hot is the link? Biochem Pharmacol 72(11):1605–1621

Auffret G, Labaied M, Frappier F, Rasoanaivo P, Grellier P, Lewin G (2007) Synthesis and antimalarial evaluation of a series of piperazinyl flavones. Bioorg Med Chem Lett 17(4):959–963

Balkwill FR, Mantovani A (2012) Cancer-related inflammation: common themes and therapeutic opportunities. Semin Cancer Biol 22(1):33–40

Barve V, Ahmed F, Adsule S, Banerjee S, Kulkarni S, Katiyar P, Anson CE, Powell AK, Padhye S, Sarkar FH (2006) Synthesis, molecular characterization, and biological activity of novel synthetic derivatives of chromen-4-one in human cancer cells. J Med Chem 49(13):3800–3808

Baylac S, Racine P (2003) Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. Int J Aromather 13(2-3):138–142

Bekir J, Mars M, Souchard JP, Bouajila J (2013) Assessment of antioxidant, anti-inflammatory, anti-cholinesterase and cytotoxic activities of pomegranate (Punica granatum) leaves. Food Chem Toxicol 55:470–475

Bhatnagar S, Sahi S, Kackar P, Kaushik S, Dave MK, Shukla A, Goel A (2010) Synthesis and docking studies on styryl chromones exhibiting cytotoxicity in human breast cancer cell line. Bioorg Med Chem Lett 20(16):4945–4950

Bray F, Jemal A, Grey N, Ferlay J, Forman D (2012) Global cancer transitions according to the human development index (2008–2030): A population-based study. Lancet Oncol 13(8):790–801

Budzisz E, Graczyk-Wojciechowska J, Zieba R, Nawrot B (2002) A new series of 2-substituted 3-phosphonic derivatives of chromone. Part II. Synthesis, in vitro alkylating and in vivo antitumour activity. New J Chem 26(12):1799–1804

do Rocio Andrade Pires A, Lecerf-Schmidt F, Guragossian N, Pazinato J, Gozzi GJ, Winter E, Valdameri G, Veale A, Boumendjel A, Di Pietro A, Pérès B (2016) New, highly potent and non-toxic, chromone inhibitors of the human breast cancer resistance protein ABCG2. Eur J Med Chem 122:291–301

Ellis GP, Shaw D (1972) Benzopyrones. 7. Synthesis and antiallergic activity of some 2-(5-tetrazolyl)chromones. J Med Chem 15(8): 865–867

Gaspar A, Matos MJ, Garrido J, Uriarte E, Borges F (2014) Chromone: a valid scaffold in medicinal chemistry. Chem Rev 114(9): 4960–4992

Gaspar A, Reis J, Kachler S, Paoletta S, Uriarte E, Klotz K-N, Moro S, Borges F (2012) Discovery of novel A3 adenosine receptor ligands based on chromone scaffold. Biochem Pharmacol 84(1): 21–29

Gaspar A, Reis J, Matos MJ, Uriarte E, Borges F (2012) In search for new chemical entities as adenosine receptor ligands: Development of agents based on benzo-g-pyrone skeleton. Eur J Med Chem 54(0):914–918

Gaspar A, Silva T, Yanez M, Vina D, Orallo F, Ortuso F, Uriarte E, Alcaro S, Borges F (2011) Chromone, a privileged scaffold for the development of monoamine oxidase inhibitors. J Med Chem 54(14):5165–5173

Gaspar A, Teixeira F, Uriarte E, Milhazes N, Melo A, Cordeiro MNDS, Ortuso F, Alcaro S, Borges F (2011) Towards the discovery of a novel class of monoamine oxidase inhibitors: structure–property–activity and docking studies on chromone amides. Chem Med Chem 6(4):628–632

Hopkins, AL, Keserü, GM, Leeson, PD, Rees DC, and Reynolds CH (2014) Nature Rev 13: 105-121.

Keri RS, Budagumpi S, Pai RK, Balakrishna RG (2014) Chromones as a privileged scaffold in drug discovery: A review. Eur J Med Chem 78(0):340–374

Khan KM, Ambreen N, Mughal UR, Jalil S, Perveen S, Choudhary MI (2010) 3-Formylchromones: potential antiinflammatory agents. Eur J Med Chem 45(9):4058–4064

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Delivery Rev 23:3–25

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65(1-2):55–63

Nam DH, Lee KY, Moon CS, Lee YS (2010) Synthesis and anticancer activity of chromone-based analogs of lavendustin A. Eur J Med Chem 45(9):4288–4292

Pan S-T, Li Z-L, He Z-X, Qiu J-X, Zhou S-F (2016) Molecular mechanisms for tumour resistance to chemotherapy. Clin Exp Pharmacol Physiol 43:723–737

Payard M (1973) Nouveau mode de préparation du chlorure d'acide chromone carboxylique-2. Bull Soc Chim Fr 7-8:2392–2394

Payard M, Paris J, Tronche P (1974) Synthèse et dissociation d'acides chromone-carboxyliques-2 et d'acides chromone-carbohydroxamiques-2. Bull Soc Chim Fr 16(3):157

Redda KK, Gangapuram M, Ghaffari MA, Eyunni S (2014) Synthesis of substituted chromone-2-phenylcarboxamides as anti-breast cancer agents. Cancer Res 74(19):1

Shimizu T, Radmark O, Samuelsson B (1984) Enzyme with dual lipoxygenase activities catalyzes leukotriene A4 synthesis from arachidonic acid. Proc Natl Acad Sci U.S.A. 81(3):689–693

Soumaoro LT, Iida S, Uetake H, Ishiguro M, Takagi Y, Higuchi T, Yasuno M, Enomoto M, Sugihara K (2006) Expression of 5-lipoxygenase in human colorectal cancer. World J Gastroenterol 12(39):6355–6360

Thanigaimalai P, Le Hoang TA, Lee K-C, Sharma VK, Bang S-C, Yun JH, Roh E, Kim Y, Jung S-H (2010) Synthesis and evaluation of novel chromone analogs for their inhibitory activity against interleukin-5. Eur J Med Chem 45(6):2531–2536

Valdameri G, Genoux-Bastide E, Peres B, Gauthier C, Guitton J, Terreux Rl, Winnischofer SMB, Rocha MEM, Boumendjel A, Di Pietro A (2012) Substituted chromones as highly potent nontoxic inhibitors, specific for the breast cancer resistance protein. J Med Chem 55(2):966–970

World Health Organization. (2013) Press release no. 223. www.molinspiration.com. Accessed 12 Dec 2013.